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Effect of Polymer Composition of Injectable Hydrogels on Programmable Release of Methylene Blue

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I. Abstract

Temperature sensitive hydrogels have been used as injectable hydrogels because the polymer solutions can be liquid at room temperature and gel at body temperature. Injectable hydrogels have been used in biomedical applications to deliver drugs and other small molecules throughout the body. Methylene Blue was used as the drug in this study for its antioxidant and neuroprotective properties. Pluronic® F127 (PF127) is a copolymer consisting of repeating units of polyethylene oxide and polypropylene oxide in the form PEO-PPO-PEO. PF127 in solution is a temperature sensitive hydrogel that transitions to a gel at body temperature at specific polymer compositions. A standard set of hydrogels was made with 17, 18, 19 and 20% composition PF127 in deionized water and 15.6 microliters of MB. Phosphate buffered saline was added to the hydrogels and measured daily for their absorbance values to determine the concentration of Methylene Blue that had been released. In an attempt to control drug release, other polymers were added to PF127 solutions. Polyethylene glycol (PEG) has been known to increase the degradation rate of compounds due to its large degree of hydrophilicity. A 1% composition of PEG was added to PF127 gels of 17 through 20% and studied for drug release. It was expected that PEG would increase the degradation rate and therefore the release rate of methylene blue. The gels with PEG behaved as expected while also increasing the gelation time. Polyvinyl alcohol was added in the amount of 1% for the opposite effect. PVA is hydrophilic like PEG, but is a polymerization stabilizer possibly due to its ability to form hydrogen bonds. Although the

PVA solution gelled at body temperature, a clump formed in the solution that could have inhibited drug release.

II. Introduction

Hydrogels have been studied for their ability to release molecules from their network of polymeric units.[1] The release properties of biodegradable hydrogels are managed by changing the porosity of the gel.[1] The size of the pores in the gel determines the molecules that can be incorporated into the gel as well as the speed at which the molecule can be released from the gel.[1] Despite the versatility of the release properties of degradable hydrogels, they must be surgically inserted into the body already in the gelatinous form.[1] The gels would have to be formed exactly to the location in the body it would be inserted, and the amorphous shapes of the body were difficult to replicate.[1] These problems were overcome by the research performed on injectable hydrogels. Because the hydrogels can be injected, it avoids the need for surgery and allows the gel to fill the defect and release desired molecules.[1] The temperature sensitivity of injectable hydrogels allow them to gel once inside the body and not at room temperature. The hydrophobic and hydrophilic regions of the gel determine the structure at different temperatures.[2] The lower critical solution temperature (LCST) is the threshold at which micelles form based on the interactions of the hydrophobic and hydrophilic blocks of the gel.[2] At any temperature above the LCST, the solution will gel.[2]

Pluronic® F-127 (PF127) is an amphiphilic, triblock copolymer consisting of polyethylene oxide and polypropylene oxide in the pattern PEO-PPO-PEO.[3,4] The gelation temperature of PF127 depends on the percent composition of polymer in solution, and the higher the percentage of polymer in solution correlates to a lower temperature required to gel.[5] From 15 to 20%, the gelation temperatures ranged from slightly above body temperature to just under room temperature.[5] PF127 was studied in a rat brain with a polymer percentage of 15% since the polymer is capable of supporting cell culture studies.[4] For this study, other polymers will be added to the PF127 solution to control the release properties of the hydrogel both to increase and decrease degradation rate, depending on the properties of the added polymer. The ideal range for this study was determined to be between 17 and 20% PF127.

Polyethylene glycol (PEG) is a hydrophilic polymer that is clear when solubilized in water. PEG comes in a variety of chain weights, ranging from 200 to 6000 Daltons.[6] PEG has been known to increase degradation of compounds as well as their solubility in water.[6] The addition of PEG to a PF127 hydrogel solution was expected to increase the degradation rate of the gel and release molecules faster.

Polyvinyl alcohol, or PVA, is a polymer that is transparent in solution and has hydrophilic properties.[7] PVA helps prevent premature degradation of polymers because of its stabilizing characteristics.[7] Because of the stabilization effects of PVA, it was expected that the addition of PVA would slow the degradation rate of PF127 and

release molecules slower.

Methylene Blue (MB) is a dark blue compound that has been used for biomedical studies as a dye or stain and for its medical applications.[8] MB has been applied to hypotension, hypoxia, and neurodegenerative diseases.[8] The neuroprotective capabilities of MB stem from the role of the compound in the mitochondria.[9] MB acts as an electron carrier in the electron transport chain, which prevents issues when the mitochondria are exposed to chemical inhibitors, specifically those that affect electron transport.[9] Methylene Blue is not affected by the chemical inhibitors, so electron transport can continue as necessary.[9] The peak absorbance wavelength for MB is 665 nanometers, which was the wavelength used in this study to collect absorbance values.[8] It was important to use hydrogels that are transparent for the drug release studies of MB so that the degraded hydrogel does not contribute to the absorbance readings.

III. Materials and Methods

Phosphate Buffered Saline Solution

Phosphate buffered saline (PBS) was used to mimic the extracellular conditions of the human body. A 10X PBS stock solution was made by combining 40 g NaCl, 1 g KCl, 7.2 g Na_2HPO_4, and 1.2 g KH_2PO_4 for a total volume of 50 mL.[10] The stock solution was diluted with 450 mL of deionized water to be used throughout testing. The concentration of the dilute PBS was 1X.

Calibration Curve

In order to determine the concentration of Methylene Blue in PBS from the absorption values, a calibration curve was made to acquire an equation that related absorption with concentration. A 20 mL vial was filled with 20 mL of deionized water and 15.6 µL of Methylene Blue. This starting solution was mixed until the MB was evenly distributed throughout the water. A baseline value was taken by putting 2 mL of pure PBS into a cuvette. For the first absorption value of the calibration curve, 2 mL of the starting solution was added to a cuvette, then the cuvette was placed in the UV-Vis spectrophotometer. The UV-Vis software gave the absorption value as the output from a wavelength of 665 nanometers. The cuvette was removed from the UV-Vis then diluted to find the next absorption value at half the starting concentration. To do this, 1 mL of the starting solution in the cuvette was removed and replaced with 1 mL of PBS. The liquid in the cuvette was mixed well by pipetting three times. The absorption value was taken by the spectrophotometer and recorded. This dilution process was repeated until the absorption value resulted in a number less than 0.1. The Beer-Lambert Law, which relates absorption and concentration linearly, is accurate if the absorption values fall between 0.1 and 1. The absorption values between 0.1 and 1, as well as their corresponding concentrations, were graphed with concentration on the horizontal axis and absorbance on the vertical axis. The linear equation from the data was used to calculate the concentration of Methylene Blue that was released from the hydrogel based on the absorbance values collected.

Hydrogels

Injectable hydrogels were created with Pluronic® F-127 (PF127) concentrations ranging from 17% to 20%, with the remaining percentage being deionized water. Stir bars and the deionized water were added to labeled 20 mL vials. For the higher percentage gels, 19% and 20%, the vials were kept at 4 ℃ while measuring the PF127 to keep the liquid cold. The cold temperature prevented the hydrogel from gelling before the polymer-water mixture was homogenized, especially since the 20% gelled at room temperature. The polymer was measured according to the percentage values, shown in Appendix A, and added to the respective vials.

The 17-20% gels containing only PF127 and water were used as the standards for this study. Another set of hydrogels consisting of PF127, water, and polyethylene glycol (PEG) represented one of the dependent variables. In addition to the 17-20% PF127, one percentage of PEG was added in place of one percent of water. The 1% PEG was first dissolved in the water before adding the PF127, then the process was the same as the standard hydrogels. This was repeated for a second dependent variable using polyvinyl alcohol. The PVA was added in the same amount, 1%, to the water. The water and PVA mixture had to be heated on a hot plate and stirred in order for the PVA to dissolve in the water. Then the PF127 was added in the amount of one percent.

The vials were transferred to the stir plate where they were stirred until the mixtures were homogenized completely. Once the gels were homogenized, 15.6 µL of Methylene

Blue was added to each and stirred again until the gels were uniform throughout. The stir bars were removed and the vials were placed back in the refrigerator until the bubbles subsided. The gels were removed from the cold and left on the bench until they reached room temperature. Each vial was observed for gelation time from room temperature and recorded. The gels were placed in an oven set at 37 degrees celsius and tested for gelation using the inversion test every 30 seconds. If the hydrogel moved at all or bubbled as a result of inversion, they were placed back in the oven and checked again after 30 seconds. Once there was no movement, the time was recorded and the vial was kept in the oven until the drug release studies so the hydrogel would remain solid.

Drug Release

Once all the gels had formed in the oven, the vials were removed and 2 mL of PBS was added to every vial then placed back in the oven. The hydrogels were kept in the oven to simulate body temperature during the drug release process. After one day, the vials were removed from the oven and 2 mL of the liquid, composed of PBS and released Methylene Blue from the gel, was placed in a cuvette and ran through UV-Vis. The 2 mL removed from the vials was replaced with 2 mL of fresh PBS. The vials were put back in the oven until the next collection time. The absorbance values were recorded, but if the values were not within the range of 0.1 to 1, 1 mL from the cuvette was removed and replaced with 1 mL of fresh PBS to dilute the liquid by a factor of two. The mixture was pipetted up and down three times to ensure that it was evenly mixed, then placed in the

UV-Vis for another absorbance reading. If the absorbance values were still not within the range, the dilution process was repeated. Absorbance value collection continued every day until all the hydrogels had degraded completely.

IV. Results

The concentration of Methylene Blue for each data point was calculated to have units of moles of MB per liter of PBS. The calibration curve was plotted as absorbance versus concentration and a line was fit to the curve.

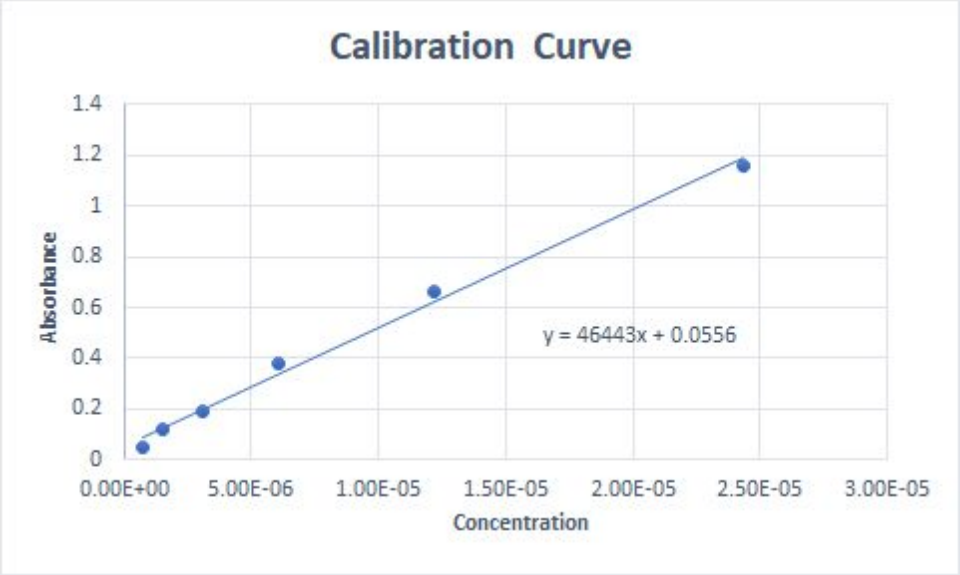

Figure 1. Calibration curve of absorbance versus concentration of methylene blue

The resulting equation for the line was $y = 46443x + 0.0556$. This equation was used to determine the concentration of released Methylene Blue for each of the hydrogels from the absorbance values.

The gelation times of the hydrogels from room temperature were recorded and graphed. Both compositions of gels were included on the graph in order to compare the percent of polymer to the time it took to completely gel.

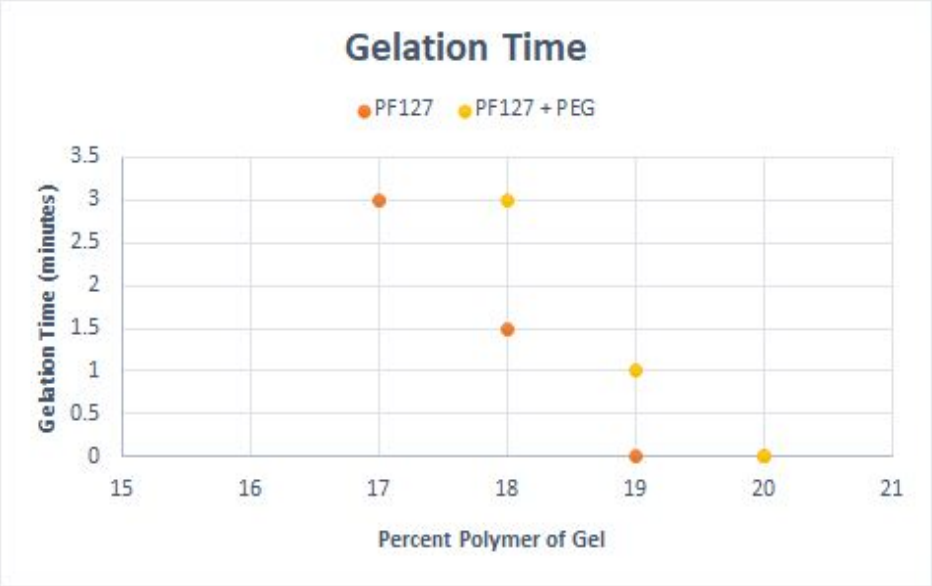

Figure 2. Gelation Time of hydrogels depending on composition

All four of the gels that were composed of only PF127 completely gelled. The only percentage that did not gel that contained both PF127 and PEG was the 17% PF127/1% PEG. The 19% PF127 gelled at room temperature, in addition to both of the 20% gels.

Cumulative release was calculated by using the equation from the calibration curve and the recorded absorbance values at each time point. The four hydrogels made of PF127 were plotted on the same graph to compare the percent release of MB to the percent of polymer of the gels.

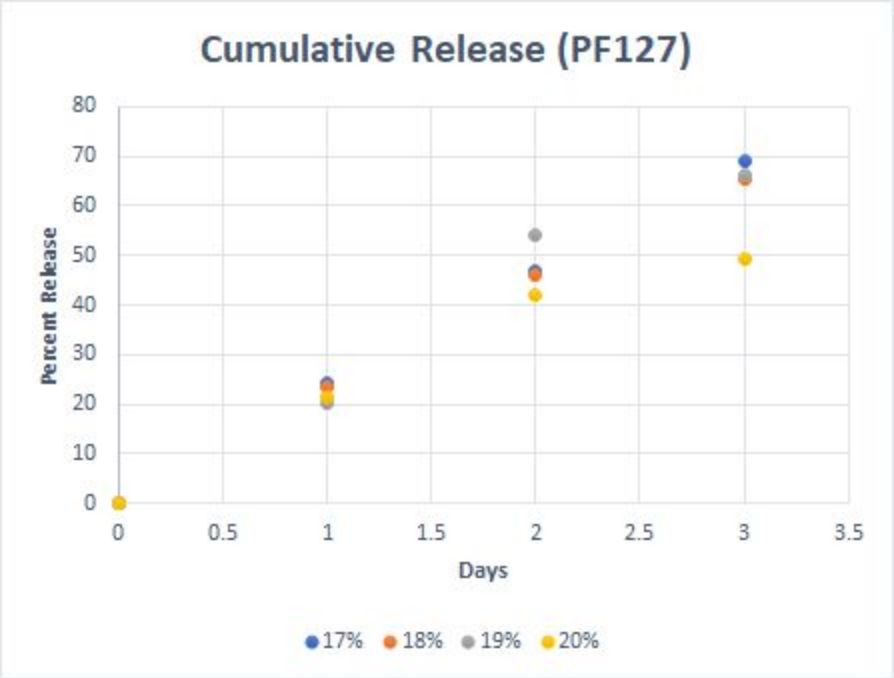

Figure 3. Cumulative Release of hydrogels made of Pluronic F-127

The hybrid hydrogels with both PF127 and PEG were also plotted on their own graph for simple comparison.

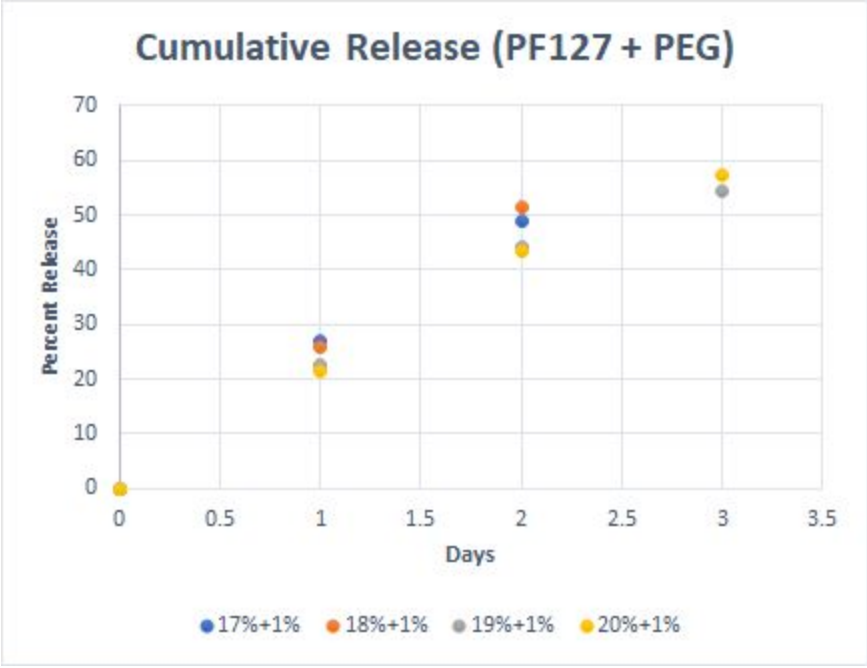

Figure 4. Cumulative Release of hydrogels made of Pluronic F-127 and polyethylene glycol

Based on the cumulative release graphs, the hydrogels consisting of a higher percentage of polymer released the drug slower over time. When comparing cumulative release of MB in regards to the composition of the polymer, PF127/PEG hydrogels released MB faster than a PF127 gel.

The hydrogels degraded over time at different rates. In the preliminary study, the gels were photographed on Day 0 and Day 7 for comparison.

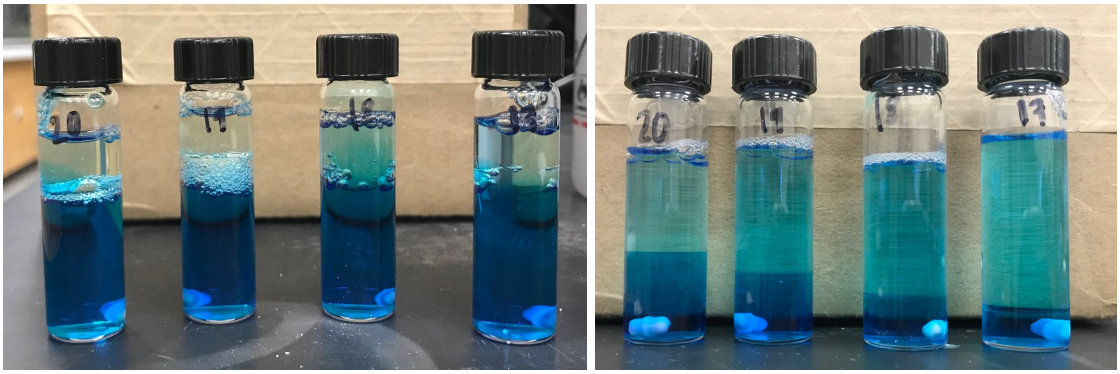

Figure 5. Day 0 versus Day 7 of drug release studies

The higher percentage hydrogels degraded slower and therefore released MB at a slower rate than the lower percentage hydrogels.

Another combination hydrogel was made of 17% PF127 and 1% PVA. The mixture of the two polymers successfully gelled.

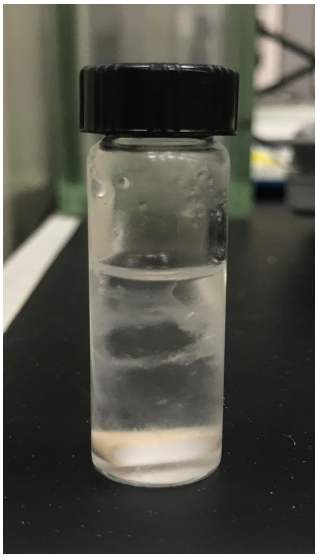

Figure 6. PF127 and PVA hydrogel with visible clump

Unfortunately, the gel had a clump that would most likely affect the delivery of drugs. The clump was more opaque than the rest of the hydrogel and was clearly visible both before and after gelation.

V. Discussion

Polyethylene glycol was shown to increase the gelation time when in reference to the standard hydrogels (PF127 gels). Figure 2 shows that for the same percentage of PF127, the hydrogel with 1% PEG required a minute longer in the oven for both 18 and 19% to gel completely. Both of the 20% gels were gelled at room temperature so the PEG did not affect that concentration. PEG may demonstrate a threshold for which it will affect the gelation of PF127 hydrogels. Because the 17% PF127/1% PEG mixture did not completely gel and the 20% PF127/1% PEG mixture was unaffected, the threshold for PEG to affect a PF127 hydrogel gelation time could possibly be between 17 and 20%.

In addition to affecting the gelation time, PEG influenced the degradation rate of the hydrogels and therefore the release rate of Methylene Blue. Figure 3 shows all of the PF127 gels fully degraded at Day 3, while in Figure 4 only two of the combination gels made it to Day 3. PEG could have contributed to increasing the rate of degradation of the gels. PF127 and PEG hydrogels release slightly more MB than PF127 at the same time points, as proven in Figures 3 and 4. The rate of Methylene Blue release increases due to the presence of PEG in the polymer. The hydrophilicity of the polyethylene glycol may be the reason for how it affects the properties of the hydrogel.

The studies with PEG were expanded upon with an attempt to add polyvinyl alcohol to a PF127 hydrogel. Although gelation of the PF127/PVA hydrogel was successful, a clump appeared that could have affected release kinetics. The PVA, being hydrophilic, could be interacting with the hydrophilic blocks of the PF127. The interaction of hydrophilic groups can determine the activity of any hydrophobic groups present on the same polymer. The hydrophilic groups may have grouped together to form a hydrophobic mass in the middle of the gel.

The surface area of the gel in contact with the PBS affected how fast the gel degraded. The 10 mL vials were used for a preliminary drug release study. A standard set of gels, consisting of only PF127, were made and studied for the release of MB. These gels degraded after over a week of absorbance values were taken, as shown in Appendix B.

On the other hand, the gels in the 20 mL vials lasted only three days. Because more of the gel was touching the PBS, the polymer degraded at a faster rate. The increase in degradation rate after exposing more of the gel to PBS may prove that PF127 breaks down via surface degradation, as opposed to bulk degradation.

VI. Acknowledgements

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VIII. Appendix

Appendix A

Table 1. Composition of Gels

Gel (% PF127)	**PF127 (g)**	**Deionized Water (mL)**	**PEG (g)**
17%	**0.85**	**4.15**	**0**
18%	**0.90**	**4.10**	**0**
19%	**0.95**	**4.05**	**0**
20%	**1.00**	**4.00**	**0**
17%PF127/1%PEG	**0.85**	**4.10**	**0.05**
18%PF127/1%PEG	**0.90**	**4.05**	**0.05**
19%PF127/1%PEG	**0.95**	**4.00**	**0.05**
20%PF127/1%PEG	**1.00**	**3.95**	**0.05**

Appendix B

Table 2. Absorbance Values of Preliminary Study with 10 mL Vials

Day	Absorbance Value (Dilution Factor of 2)
0	0
1	0.825
2	0.829
3	0.915
4	0.835
6	0.787
7	0.770
8	0.742
9	0.633